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Distribution of H Type 1 and H Type 2 Antigenic Determinants in Human Sera and Saliva

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SUMMARY

A radioimmunoassay specific for the H type 1 antigenic determinant demonstrated that the H type 1 antigen is under the strict control of the *Se* gene in both serum and saliva. Similar amounts of H type 1 antigenic determinants were found in saliva from *Se/-*, *le/le* donors and in saliva from *Se/-*, *Le/-* donors. However, sera from *Se/-*, *le/le* donors were about 100 times more efficient in inhibiting the H type 1 assay than were sera from *Se/-*, *Le/-* donors.

A radioimmunoassay, based on the binding of *Ulex europaeus* with the H type 2 antigenic determinant, showed that all the H type 2 antigen in saliva is under the control of the *Se* gene, while only one-third of the H type 2 antigen present in serum is under the control of this gene. The remaining two-thirds of H type 2 antigen in sera is independent of the ABH secretor status of the donor. The amount of H type 2 antigen in both serum and saliva is independent of the *Le* gene.

These results are compatible with the existence of two $\alpha(1\rightarrow2)$ fucosyltransferases but suggest that the enzyme of epithelial origin, coded by the *Se* gene, should be able to transform both type 1 and type 2 natural substrates, while the enzyme of mesodermic origin, coded by the *H* gene, would work preferentially on the natural type 2 substrates.

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INTRODUCTION

The immunodominant sugar of the H antigenic determinant is an L-fucose-linked $\alpha(1\rightarrow2)$ to the terminal βD-galactose of the ABH blood group precursor chains. The possible existence of two $\alpha(1\rightarrow2)$ fucosyltransferases was first suggested [1] on the basis of stereochemical differences that exist between the two precursor chains, type 1 (βDGal(1→3)βDGlcNAc . . . R) and type 2 (βDGal(1→4)βDGlcNAc . . . R) (fig. 4). The genetic analysis of *H*-deficient pedigrees (Bombay) strongly supported a two-structural gene model, *Se* and *H*, each coding for a different $\alpha(1\rightarrow2)$ fucosyltransferase [2]. This model differs from the generally accepted model [3] by the fact that the *Se* gene would be a structural gene instead of a regulatory gene controlling the expression of the structural gene *H*.

Glycosphingolipids with ABH and Lewis antigenic determinants have been found in sera [4], and they have been successfully transferred from sera to red cells [5–8] and to lymphocytes [8–11]. The expression of these soluble ABH and Lewis glycosphingolipids is controlled by the secretory system. As opposed to this, the ABH antigens synthesized by red cell precursors and vascular endothelia are known to be independent of the secretory system. Based on the determination of ABH and Lewis groups in different tissues of bone marrow graft donors and recipients, we proposed that the H antigen of epithelial origin (found in secretions, sera, lymphocytes, and red cells) could be synthesized by an enzyme coded by the *Se* gene, while the H antigen of mesodermic origin (found in vascular endothelia and red cells) could be synthesized by a different enzyme coded by the *H* gene [11].

Here, radioimmunoassays specific for H type 1 and H type 2 antigenic determinants are described and the expected properties of the enzymes produced by the postulated structural genes *H* and *Se* are discussed according to the relative amounts of H type 1 and H type 2 antigens found in saliva and serum.

MATERIALS AND METHODS

Artificial Antigens

Synthetic oligosaccharides were coupled to bovine serum albumin (BSA) at a molar hapten/BSA ratio of 15–20 to 1 [1]. The structures of the synthetic oligosaccharides are shown in figure 1, and the details of their chemical synthesis will be given elsewhere (Lemieux et al., in preparation). The hapten-BSA compounds were labeled with ^{125}I ($\simeq$ 10 μC/μg) by the chloramine T method [12].

Immunoabsorbents

The same synthetic oligosaccharides used to prepare the artificial antigens were coupled to insoluble matrices of crystalline silica (cristobalite) [1]. These immunoabsorbents are now commercially available under the trade name of Synsorb, from Chembiomed (The University of Alberta, Edmonton, Canada).

H Type 1 Reagent

Anti-H serum was obtained from goats hyperimmunized with crude saliva from *O/O, le/le, Se/-* donors in complete Freund adjuvant. The serum was run through an H type 2 immunoabsorbent column to remove antibodies crossreacting with H type 2 antigenic determinants. The antibodies remaining, in the effluent, were then bound to an H type 1

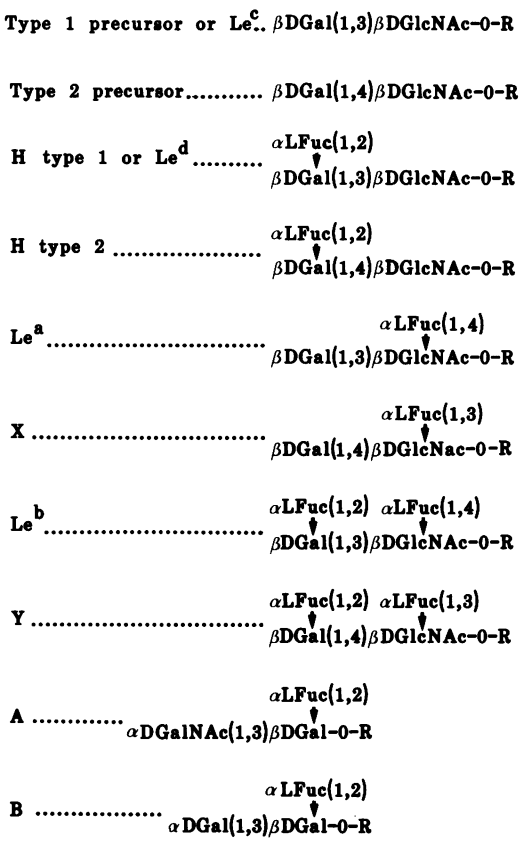

FIG. 1.—Synthetic oligosaccharides used to define the specificity of H type 1 and H type 2 reagents. Abbreviations: Gal = galactose; GalNAc = *N*-acetylgalactosamine; GlcNAc = *N*-acetylglucosamine; Fuc = fucose; R = $(CH2)_8$-COOH.

immunoabsorbent column. The column was thoroughly washed with phosphate buffered saline, and the specific H type 1 antibodies were eluted with 1% NH_4OH in 0.15 M NaCl. The yield of refined H type 1 antibodies was 70%.

H Type 2 Reagent

Ulex europaeus lectin 1 purified by affinity chromatography was obtained from Pharmindustrie IBF (35 avenue Jean Jaurès, 92390 Villeneuve-la-Garenne, France).

Human Saliva and Serum Samples

Saliva and serum were obtained from normal blood donors (identified with numbers) and kidney patients, either transplanted or waiting for a kidney transplant (identified with letters). Saliva samples were heated in a boiling water bath for 10 min within 1 hr of collection, and then were clarified by centrifugation at 5,000 *g* for 10 min. Both sera and saliva samples were stored frozen at −20°C until used. Frozen sera samples from two Bombay Le(a+b−) donors (expected to be *h/h, se/se* according to the model proposed in [2]) were obtained from J. P. Cartron (CNTS, Paris, France).

Blood Groups

The ABO and Lewis phenotypes of serum and saliva donors were determined with conventional hemagglutination techniques. To avoid any differences due to the ABO system, only blood group O donors were selected for the present study. However, preliminary results showed that serum and saliva from A and B donors give similar inhibition patterns in both H type 1 and H type 2 assays as the serum and saliva from O donors (our unpublished results, 1981).

The ABH secretory status was determined in saliva by inhibition of hemagglutination using the purified lectin 1 from *Ulex europaeus*. Genotypes were inferred from phenotypes assuming that *A* and *B* are codominant to *O*, *Se* is dominant to *se*, and *Le* is dominant to *le*.

H Type 1 Assay

One-ml polystyrene tubes were coated overnight with 50 μl of a 5 μg/ml solution of purified H type 1 antibodies. After washing, 50 μl aliquots of the twofold serial dilutions of the inhibitors in 1% BSA were added. The tubes were incubated overnight at room temperature and washed. Fifty μl of the H type 1-BSA^{125}I antigen solution (≃ 10,000 cpm), in cold 1% BSA, was added, and the tubes were again incubated overnight. After washing, the bound radioactivity was counted in an LKB automatic gamma counter. In absence of inhibitor, 40% of the radioactivity was bound to the coated tubes. All washings were performed with 1 ml of tap water, and each point was carried out in triplicate.

H Type 2 Assay

This assay was similar to the H type 1 assay, but the tubes were coated with a 10 μg/ml solution of the purified lectin 1 from *Ulex europaeus*, and the incubation, with the H type 2-BSA^{125}I artificial antigen, was stopped at 3 hrs. Under these experimental conditions, 35% of the total radioactivity added (≃ 10,000 cpm) was bound to the coated tubes in the absence of inhibitor.

Although strongly circumstantial evidence exists that the H type 2 structure may be present in salivas, we have no knowledge of a direct proof. The combining site of *Ulex europaeus* lectin 1 is known to not necessarily be restricted to this structure (for a review, see [13]). Nevertheless, for reasons of convenience and the strong possibility that this assay is in fact assessing the presence of H type 2 antigens, the assay is termed an H type 2 assay. Our treatment of the results obtained is made accordingly. Should this assumption prove not to be correct, then the conclusion would be that neither secretors nor nonsecretors secrete H type 2 antigens in their salivas. The nature of the *Ulex* active substance that is present in the salivas of secretors would then have to be established.

Calculations

The percent inhibitions of binding of the ^{125}I-labeled artificial antigens were plotted in a semilogarithmic scale. The best fitted straight lines between 30% and 70% inhibition were calculated by the least-squares method. Two parameters were considered throughout: (1) the dilution of saliva or serum necessary to inhibit 50% of the binding of the ^{125}I-labeled artificial antigen, and (2) the slopes of the calculated straight inhibition lines. When the slopes of the inhibition lines are parallel, the dilutions that provide 50% inhibition are related to the relative amounts of antigen in the samples. When the slopes are different, quantitative comparisons of antigen contents cannot be performed. However, since the slope of an inhibition curve is a function of the binding constant of the system [14], within a given assay, the antigen that provided the steepest slope is expected to best bind the corresponding antibody or lectin.

RESULTS

Specificity of the H Type 1 or Le^d Assay

Of the artificial antigens tested, the binding of the H type 1-BSA^{125}I antigen to the coated anti-H type 1 tubes was strongly inhibited only by the cold H type 1-BSA antigen. Fifty percent inhibition was obtained with 40 ng/ml, and the slope was of −0.8 (fig. 2). A flat inhibition curve, indicating lower binding constant, was obtained with cold Le^b-BSA antigen, and no significant inhibition was detected with either H type 2-BSA, Y-BSA, X-BSA, Le^a-BSA, type 1 precursor-BSA (Le^c-BSA), type 2 precursor-BSA, B-BSA, or A-BSA (see fig. 1 for the structures of these antigens).

Specificity of the H Type 2 Assay

The binding of the H type 2-BSA^{125} antigen to the *Ulex europaeus*-coated tubes was strongly inhibited only with the cold H type 2-BSA antigen. Fifty percent inhibition was obtained with 100 ng/ml, and the slope was of −2.1 (fig. 3). Some inhibition, with a weak slope, was observed with the Y-BSA antigen, and no specific inhibition could be detected with either Le^a-BSA, Le^b-BSA, type 1 precursor-BSA (Le^c-BSA), Le^d-BSA (H type 1-BSA), A-BSA, B-BSA, X-BSA, or with type 2 precursor-BSA.

Assay of H Type 1 Activity in Human Serum

All the samples from secretors inhibited the H type 1 assay (table 1). No significant inhibition could be detected with the sera from nine Lewis positive nonsecretor donors, with the sera of five Lewis negative nonsecretor donors, or with the sera of two Bombay Le(a+b−) individuals. The inhibition curves obtained using

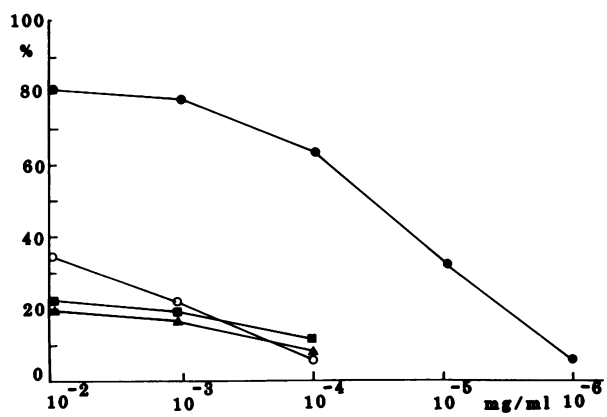

FIG. 2.—Inhibition of binding of H type 1-BSA^{125}I to anti-H type 1 by artificial antigens. Artificial antigens: H type 1-BSA (●); H type 2-BSA (▲); Le^b-BSA (○); Y-BSA (■). All the other chemically related artificial antigens tested (type 1 precursor-BSA, type 2 precursor-BSA, Le^a-BSA, X-BSA, A-BSA, and B-BSA [not shown in fig. 2]) did not give detectable inhibition. *Vertical axis* = % inhibition of binding. *Horizontal axis* = antigen dilution mg/ml.

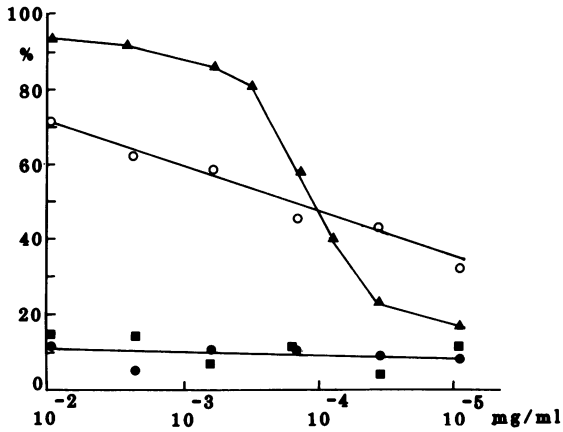

FIG. 3.—Inhibition of binding of H type 2-BSA^{125}I to *Ulex europaeus* lectin by artificial antigens. Artificial antigens: H type 2-BSA (▲); H type 1-BSA (●); Y-BSA (○); Le^a-BSA (■). The remaining artificial antigens tested (type 1 precursor-BSA, type 2 precursor-BSA, X-BSA, Le^b-BSA, A-BSA, and B-BSA [not shown in fig. 3]) did not show specific inhibition. *Vertical axis* = % inhibition of binding. *Horizontal axis* = antigen dilution mg/ml.

the sera of either Lewis positive or Lewis negative secretors had similar slopes (1 ± 0.1). However, as seen from table 1, the sera from Lewis negative secretor donors (770 ± 190) were about 100 times more efficient as inhibitors than were the samples from Lewis positive secretor donors (5 ± 1).

Assay of H Type 2 Activity in Human Serum

In contrast to the above-described H type 1 assay, as expected, all normal sera samples, irrespective of ABH secretor status, inhibited the H type 2 assay. The slopes of the inhibition lines were similar, and the dilutions necessary for 50% inhibition were of the same order of magnitude for the four genotypes analyzed (table 2). However, the average dilution for 50% inhibition of ABH secretors (14 ± 1) was significantly higher than that of ABH nonsecretors (9 ± 1) ($P < .01$), suggesting that secretors have some 30% more H type 2 antigen in serum than do nonsecretors. The sera of the two Bombay individuals were the only samples that did not inhibit the H type 2 assay.

Assay of H Type 1 Activity in Human Saliva

No inhibition could be detected with the samples of eight salivas from nonsecretors. Alternatively, all salivas of secretors strongly inhibited the H type 1 assay (table 3). The slopes were similar for both Lewis positive secretor donors (−2.2 ± 0.4) and Lewis negative secretor donors (−2.1 ± 0.3). The average dilution for 50% inhibition of the samples from Lewis negative secretor donors (8,300 ± 2,460) was higher than the average dilution of samples from Lewis positive secretor donors (4,240 ± 1,320), but the difference was not statistically significant (table 3).

TABLE 1

INHIBITION OF BINDING OF H TYPE 1-BSA^{125}I TO ANTI-H TYPE 1 BY NORMAL BLOOD GROUP O SERA*

Secretor and Lewis genotype	Donor identification	Dilution of sera for 50% inhibition*	Slope of inhibition lines
Se/-, le/le	CAL	1800	−1.0
	4755	1000	−1.0
	KOB	650	−1.0
	4484	650	−1.0
	3992	500	−0.9
	4246	400	−1.0
	4290	400	−0.9
Average ± SE.....		700 ± 190	−1.0 ± 0.1
Se/-, Le/-	6519	7	−1.0
	4046	6	−0.9
	4490	5	−1.1
	4025	5	−1.1
	6676	5	−1.1
	5324	5	−1.3
	4835	5	−1.2
	5160	4	−1.1
	6256	3	−1.1
	3943	2	−0.8
Average ± SE.....		5 ± 1	−0.8 ± 0.1

NOTE: Sera from two Bombay and 14 normal nonsecretors produced no detectable inhibition irrespectively of their Lewis type.

**Se/-, le/le* vs. *Se/-, Le/-*; $P < .001$ ($t = 4.95$).

Assay of H Type 2 Activity in Human Saliva

The H type 2 inhibition pattern of the four combined Lewis and secretor genotypes was almost identical with the inhibition pattern given by salivas in the radioimmunoassay for the H type 1 determinant. The samples from both Lewis positive and Lewis negative ABH secretors gave strong inhibitions with similar slopes (2.1 ± 0.1 and 1.8 ± 0.1) and similar 50% inhibition. The samples from 15 nonsecretors did not significantly inhibit the system.

In parallel to the H type 1 system, the amount of H type 2 activity in saliva was independent of the Lewis genotype of the donor (table 4).

DISCUSSION

The inhibitions (figs. 2 and 3) performed with 10 artificial antigens (fig. 1) showed that both systems were specific for the corresponding type 1 and type 2 chains. In each system, the corresponding monofucosylated H antigen was the best inhibitor. Some cross reaction was observed with the difucosylated Le^b-BSA in the H type 1 assay and with the difucosylated Y-BSA in the H type 2 assay. In both instances, the cross reaction with the difucosylated structures were weak and the inhibition plots showed no inflection suggesting low binding constants. The results of the H type 2 assay are in agreement with the reported specificity of lectin 1 from *Ulex europaeus* [15].

The inhibition curves, from different donors of the same genotype, were parallel within the same biological fluid, but saliva had consistently steeper inhibition slopes than did sera in both H type 1 and H type 2 assays (tables 1–4). Differences in size of the antigen carrier molecule, and/or in the number and the distribution of the antigenic determinants at the surface of the corresponding carrier, might explain the different slopes given by serum and saliva. Since these parameters, and their eventual relationship with the artificial antigens, are not well defined, the

TABLE 2

INHIBITION OF BINDING OF H TYPE 2-BSA^{125}I TO *Ulex europaeus* BY NORMAL BLOOD GROUP O SERA*

Secretor and Lewis genotype	Donor identification	Dilution of sera for 50% inhibition*	Slope of inhibition lines
Se/-, le/le	KOB	29	−0.7
	4755	16	−0.8
	CAL	14	−0.8
	4484	12	−0.7
	3992	11	−0.6
	4246	6	−0.8
Average ± SE		14 ± 3	−0.7 ± 0.1
Se/-, Le/-	FEN	24	−0.9
	PAN	21	−0.8
	4122	14	−0.8
	4364	14	−0.9
	5170	14	−0.9
	MIG	12	−0.6
	TOU	12	−0.8
	4811	11	−0.9
	ZIT	11	−0.7
	LAN	9	−0.7
Average ± SE		14 ± 1	−0.8 ± 0.1
se/se, Le/-	3979	15	−0.9
	4221	13	−0.8
	4752	13	−0.8
	4151	10	−0.9
	GAY	10	−0.8
	HAK	10	−0.8
	HUW	7	−0.9
	MEU	6	−0.6
	CAZ	5	−0.9
	TAI	4	−0.8
Average ± SE		9 ± 1	−0.8 ± 0.1
se/se, le/le	4836	10	−0.8
	5097	8	−0.7
	4748	8	−0.7
	4813	7	−0.7
Average ± SE		8 ± 1	−0.7 ± 0.1

NOTE: Sera from two Bombay produced no detectable inhibition.
* *Se/-* vs. *se/se*; $P < .01$ ($t = 3.08$).

TABLE 3

INHIBITION OF BINDING OF H TYPE 1-BSA^{125}I TO ANTI-H TYPE 1 BY NORMAL BLOOD GROUP O SALIVAS

Secretor and Lewis genotype	Donor identification	Dilution of sera for 50% inhibition*	Slope of inhibition lines
Se/-, le/le	MET	20000	−2.4
	GUI	15000	−2.4
	HUS	11000	−2.2
	VAU	10000	−2.4
	CAU	6100	−2.1
	LAH	1600	−2.0
	SIC	1600	−1.6
	DAN	1100	−2.0
Average ± SE.....		8300 ± 2460	−2.1 ± 0.1
Se/-, Le/-	TEX	13000	−2.2
	GUI	8700	−2.6
	LEL	7500	−2.9
	VIT	4200	−2.5
	LEN	3000	−1.9
	VAG	2000	−2.0
	LEG	1500	−2.2
	GUL	1200	−1.9
	LES	1100	−1.6
	LEB	160	−1.8
Average ± SE.....		4240 ± 1320	−2.2 ± 0.1

NOTE: Saliva from nonsecretors (*se/se, Le/-* and *se/se, le/le*) produced no detectable inhibition.
**Se/-, le/le* vs. *Se/-, Le/-*; $.1 < P < .2$ ($t = 1.54$).

dilution giving 50% inhibition can be considered only as a relative estimate of the amounts of antigen present in a given biological fluid.

Genetic Control of H Type 1 Antigen

Le^d and Le^c are used as synonyms of H type 1 and type 1 precursor, respectively, according to the genetic model proposed by Graham et al. [16]. Although the chemical structure of the Le^c antigenic determinant is still controversial, the type 1 chemical structure of the Le^d or H type 1 antigen is already well documented [17, 18].

The H type 1 antigen was detected only in the sera or saliva of ABH secretors. Therefore, the synthesis of H type 1 is under the strict control of the *Se* gene.

A new and unexpected finding of this study was the Lewis-related difference observed between sera and saliva. Table 3 shows insignificantly different amounts of H type 1 in saliva of Lewis negative secretor and Lewis positive secretor donors. On the other hand, as seen in table 1, there is a 100-fold difference in the amount of H type 1 antigen present in sera of *Lewis negative* secretor donors as compared with *Lewis positive* secretor donors. These results require that the amount of H type 1 antigenic determinant in sera of Le^b donors is very small. Indeed, large amounts of Le^b antigen (our unpublished results, 1981) and very little H type 1 antigen (table 1) were found in sera from Le^b individuals by radioimmunoassay.

This is in contrast with the findings in saliva, where a large fraction of the same H type 1 determinants seems to remain unconverted to the Le^b determinant by the Lewis transferase, showing that, for some unknown reason, this last enzyme is less efficient than is the H transferase in certain compartments of the body. When the $\alpha(1\rightarrow2)$ and the $\alpha(1\rightarrow4)$ fucosyltransferases, present in epithelial homogenates, were allowed to compete in vitro for a limited amount of substrate, the Lewis transferase was 10 [19] or 100 [20] times more efficient than the $\alpha(1\rightarrow2)$ fucosyltransferase, showing that the experimental conditions of the in vitro enzyme studies are not the exact reflection of the reactions taking place in vivo.

TABLE 4

INHIBITION OF BINDING OF H TYPE 2-BSA^{125}I TO *Ulex europaeus I* LECTIN BY NORMAL BLOOD GROUP O SALIVAS

Secretor and Lewis genotype	Donor identification	Dilution of sera for 50% inhibition	Slope of inhibition lines
Se/-, le/le	GUI	5800	−1.4
	ORL	5800	−1.9
	LIA	5400	−2.5
	MET	5400	−1.9
	LAH	3900	−1.8
	HUS	3800	−1.5
	VAN	3800	−1.8
	MAC	3800	−2.1
	VAU	3500	−2.3
	MUN	3500	−1.9
	CAU	2600	−1.5
	MAR	1600	−1.4
	SIC	1000	−1.9
	WAN	550	−1.3
	DAN	300	−1.6
Average ± SE		3380 ± 480	−1.8 ± 0.1
Se/-, Le/-	LEG	12000	−1.4
	GUI	10000	−2.1
	TEX	8800	−2.7
	IZZ	7000	−2.1
	DAI	5400	−1.3
	COI	5100	−3.1
	JEA	3000	−1.7
	JAC	2700	−2.1
	JUL	2400	−2.3
	DAR	1900	−1.8
	SAN	1900	−1.8
	JEU	1800	−1.9
	VAG	1100	−2.4
	GUI	800	−2.1
	LEG	800	−2.6
	LES	700	−2.0
	LEN	700	−2.0
Average ± SE		3890 ± 870	−2.1 ± 0.1

NOTE: Saliva from nonsecretors (*se/se, Le/-* and *se/se, le/le*) produced no detectable inhibition.

Genetic Control of the H Type 2 Antigen

The letters X and Y have been used [21] to define the fucosylated structures on type 2 chains analogous to the Le^a and Le^b antigens on type 1 chains (fig. 1).

As opposed to the H type 1 antigen, which seems always under the control of the *Se* gene, a double genetic control seems to operate on the synthesis of the H type 2 antigen. About two-thirds of the H type 2 antigen in sera was independent of the *Se* gene (table 2), while in saliva, all the H type 2 antigen found was under the control of the *Se* gene (table 4). A second difference between H type 1 and H type 2 is that the expression of H type 2 was always independent of the *Le* gene (tables 2 and 4), whereas the amounts of H type 1 antigen were always higher in *Se/-*, *le/le* than in *Se/-*, *Le/-* (tables 1 and 3). This is not surprising since the formation of the Le^b determinant is at the expense of the H type 1 precursor.

How Many H Enzymes Are There?

Our original two-structural-gene model proposed the existence of two H enzymes [2]. Although there exists no direct biochemical proof, some evidence points in this direction. The $\alpha(1\rightarrow2)$ fucosyltransferase found in milk, submaxillary glands, and stomach mucosa occurs only in ABH secretors [22]. This is in contrast with the $\alpha(1\rightarrow2)$ fucosyltransferase found in sera that is present in all ABO donors irrespective of their ABH secretor status [22, 23]. Moreover, our present results show that the sera of Lewis negative and Lewis positive *nonsecretors* have no H type 1 activity (see results of H type 1 assay in sera). However, a normal type 1 precursor glycolipid has been found in the *serum* of a Lewis negative donor, and this type 1 acceptor was successfully transformed in vitro with gastric mucosa enzymes into Le^a and Le^b [24]. Since in order to produce Le^b it is necessary to add the $\alpha(1\rightarrow2)$L-fucose of the H type 1 determinant, these results strongly suggest that the $\alpha(1\rightarrow2)$ fucosyltransferases present in sera and in gastric mucosa have different acceptor requirements and hence are different.

Expected Properties of the Two $\alpha(1\rightarrow2)$ Fucosyltransferases Coded by the Se *and the* H *Genes*

In our original model, we proposed that the enzyme coded by the *Se* gene (found in epithelial tissue) could be specific for type 1, and the enzyme coded by the *H* gene (found in mesodermic tissue) could be specific for type 2 substrates [2]. The present results are compatible with the existence of two enzymes, but they suggest that the enzyme coded by the *Se* gene might be able to transform both type 1 and type 2 substrates, while the enzyme coded by the *H* gene might be restricted to the type 2 substrate. This modification of the original model can account for the presence of large amounts of H type 2 antigen in saliva of secretors, and for the increased expression of type 2, subjacent I antigen in saliva [25] and stomach [26] of nonsecretors. The present results show that, in the case of secretors, about one-third of the H type 2 antigen, present in sera, may come from secretory glands and epithelia (under the control of the *Se* gene). The remaining two-thirds of the H type 2 antigen in sera may be released into circulation by endothelial or bone

marrow cells (independent of the *Se* gene). The new variant of the genetic model is also compatible with the conformational analysis of the type 1 and type 2 precursor structures. As shown in figure 4, the shielding of the 2'hydroxyl group of the galactosyl residue on the type 1 precursor by the acetyl group of the glucosamyl residue could refrain a type 2 specific $\alpha(1\rightarrow2)$ fucosyltransferase, coded by the *H* gene, from using this precursor as an acceptor. On the other hand, the absence of shielding of the equivalent 2'hydroxyl group in the type 2 precursor would allow a type 1 $\alpha(1\rightarrow2)$ fucosyltransferase coded by the *Se* gene to use both type 1 and type 2 precursors as acceptors.

Previous in vitro observations [22] of $\alpha(1\rightarrow2)$ fucosyltransferase activity, from secretory epithelia, have shown formation of both H type 1 and H type 2 structures. However, the same study [22] also showed formation of both H type 1 and H type 2 structures with H enzyme from serum. As mentioned above, it is possible that the in vitro experiments using disaccharides as acceptors do not show the same acceptor specificity, or regulation mechanisms, as does the in vivo transfer with natural acceptors.

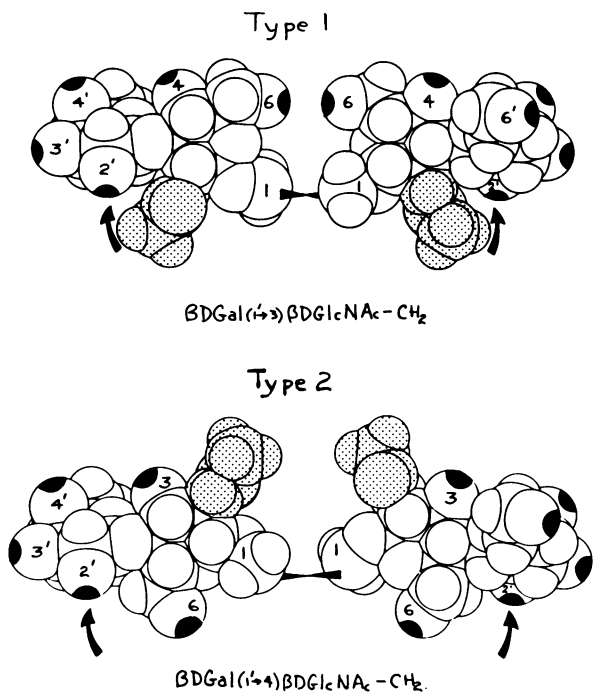

FIG. 4.—Comparison of the accessibility to the 2'hydroxyl group (*arrows*) of the terminal galactose in type 1 and 2 precursor chains. The acetyl group of the subterminal glucosamine (*shaded area*) restricts the access to the 2'hydroxyl group in type 1, whereas it leaves a different and freer access to the same hydroxyl group in type 2. The frontal projection (*left*) was turned 180° around the C1 of glucosamine (⋈) to get the back projection (*right*) of the three-dimensional model obtained by hard sphere calculations (HSEA).

Type 1 and type 2 chains have been described in glycolipids and glycoproteins from secretions of humans and mammals (reviewed in [27]), while the type 2 antigens of red cells and vascular endothelia have been found only in man and some anthropoid apes.

If our two-structural-gene model is correct, we can speculate that the ancestral gene, present in mammals, is the equivalent of the *Se* gene and that the duplication event that originated the new gene *H* in apes involved a restriction on the acceptor specificity of the enzyme and that this new enzyme, coded by the gene *H*, had lost the capacity to transform the type 1 acceptor and works preferentially on the type 2 natural acceptors.

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